nature OUTLOOK

CANCER IMMUNOTHERAPY

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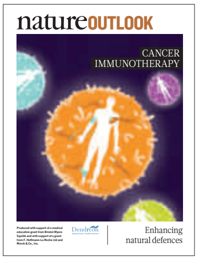

Cover art: Neil Webb

The past few decades have seen a groundswell of research on the immune system yielding a deeper understanding of how cancer progresses and offering new ways to stop it. As this Outlook reports, a range of cancer therapies that exploit the complex interactions between tumours and immune cells are the result of this effort.

Cancer immunotherapy is the product of reciprocal learning: even as the immune system teaches researchers about its intricacies, researchers are teaching immune cells how to use those abilities to specifically target disease. By using what they learn about the immune system, scientists can create synthetic molecules to attack a tumour, or can help the immune system to do its job more effectively (S2).

In 1891, William Coley injected cancer patients with bacteria to ignite an immune response — a strategy now experiencing a revival (S4). Immunologists are finding ways to harness the immune system, including training immune cells to recognize a patient's particular cancer (S9 and S13).

The finding that tumours can actively suppress immunity has led to the development of 'checkpoint blockades' that prevent this suppression (S6). Medical imaging technology is providing a clearer picture of how cancerous cells interact with other cells, even at the molecular level (S10). Bioengineers are getting involved, too: the first implantable cancer vaccine, made by combining the latest cancer immunology with materials science, entered clinical trials in 2013 (S16). And patients are already benefiting, as Carley Rutledge — a Colorado student — attests in a naturevideo that accompanies this Outlook (go.nature.com/yfvwwi).

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Lauren Gravitz
Contributing Editor

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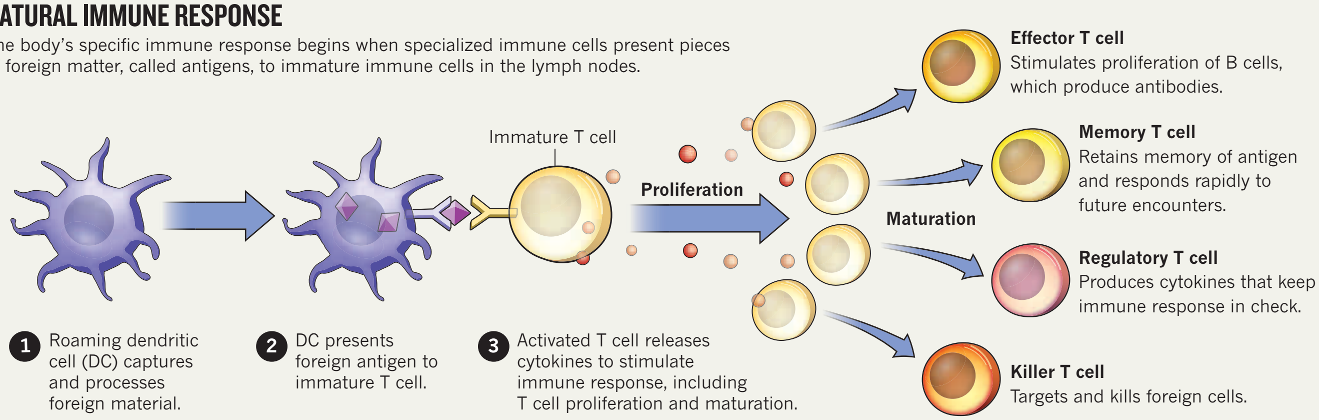
CALLING CELLS TO ARMS

Increased understanding of immune- and tumour-cell biology has led to an explosion of research into potential ways to harness the immune system to kill cancer. By **Emily Elert**.

NATURAL IMMUNE RESPONSE

The body's specific immune response begins when specialized immune cells present pieces of foreign matter, called antigens, to immature immune cells in the lymph nodes.

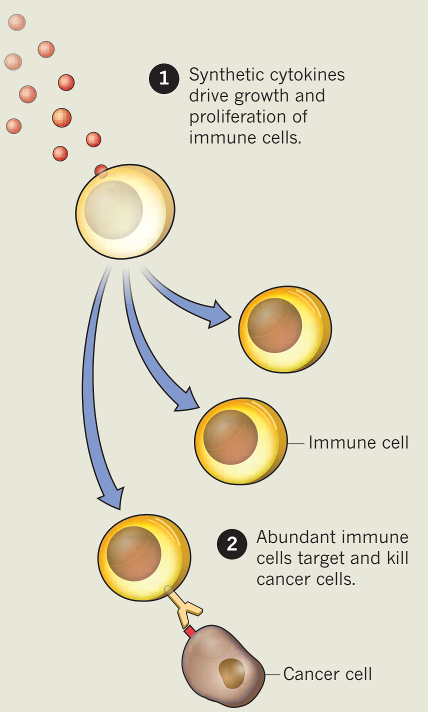


THERAPUTIC APPROACHES

Current cancer immunotherapies can be broken down into three major types: non-specific therapies, monoclonal antibodies and vaccines.

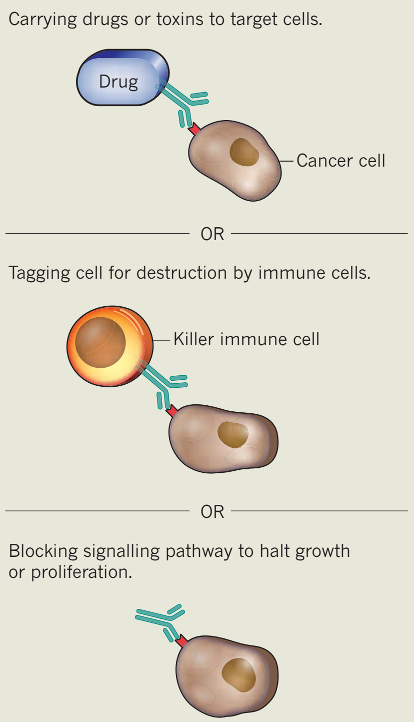
NON-SPECIFIC IMMUNOTHERAPIES

These drugs include cytokines and other chemicals that stimulate a general immune response. Most likely to be used as adjuvants to other therapies, such as vaccines.



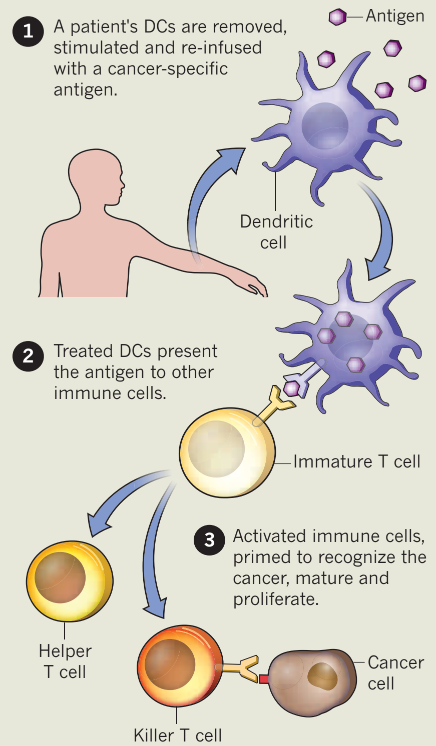
MONOCLONAL ANTIBODIES

These proteins stick to specific antigens, either directly affecting cells or tagging them for destruction. Uses include:



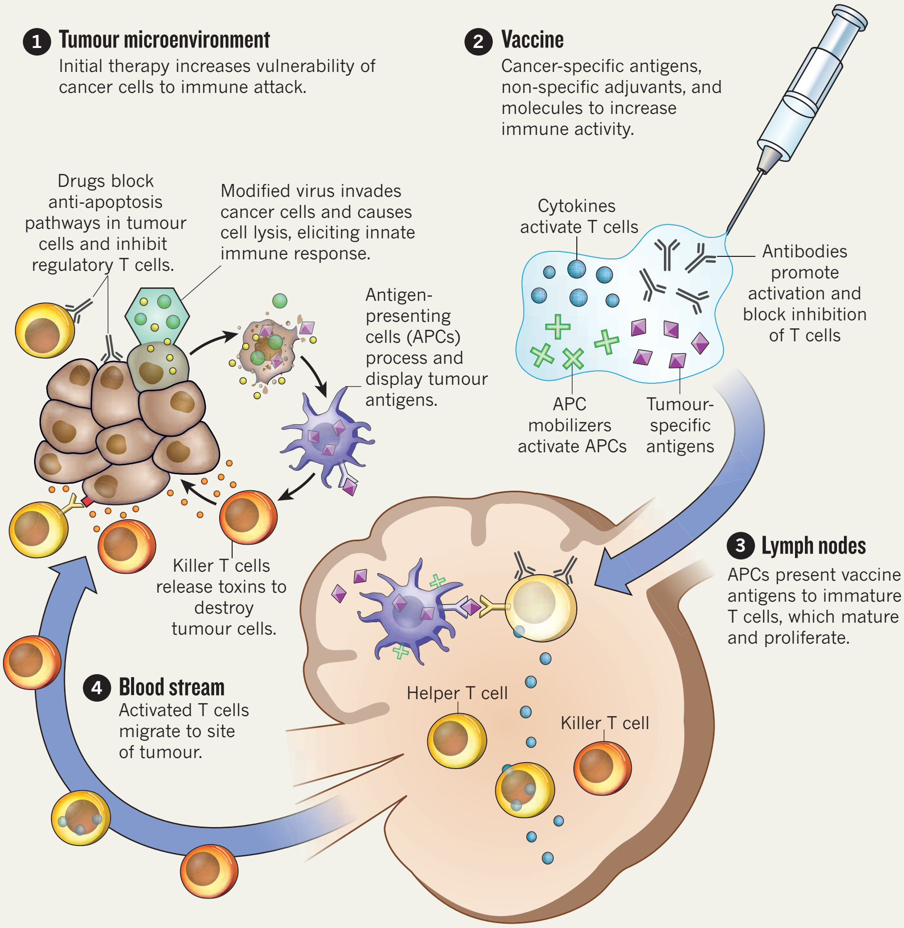
VACCINES

Vaccines are made from cancer cells, parts of cells or antigens designed to stimulate the immune system to attack a tumour. Multiple approaches are being tested, including DC vaccines.



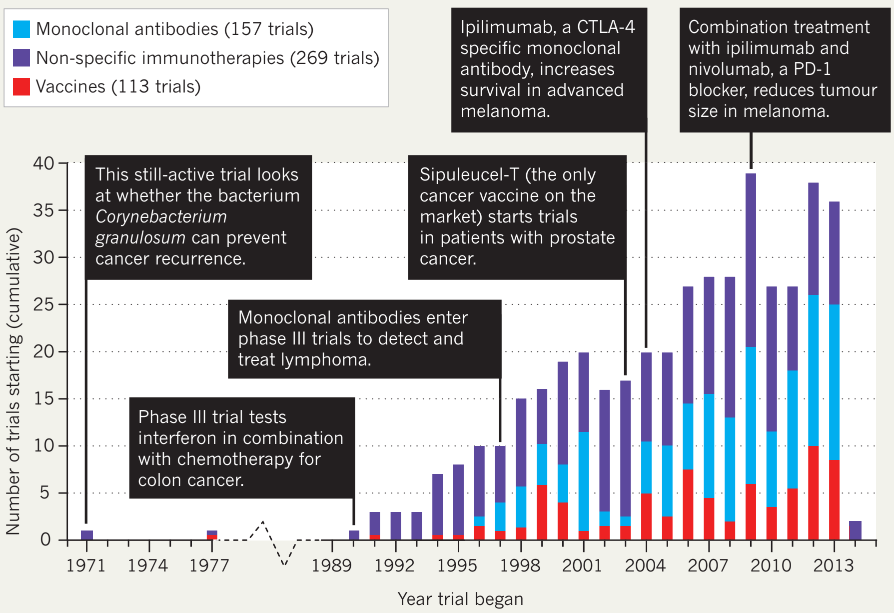
SEARCHING FOR SYNERGY

Single immunotherapies have been only modestly effective, so researchers are searching for synergistic combinations of drugs. The ideal attack below includes existing therapies, drugs in clinical trials and theoretical compounds.



IMMUNOTHERAPIES ON TRIAL

The number of cancer immunotherapies in phase III clinical trials has risen sharply since the early 1990s, reflecting renewed interest in immune-based cancer treatments among researchers and drug-makers.



A DURABLE CONCEPT

1891 After reading that a patient's tumour disappeared after a bacterial infection, surgeon William Coley begins injecting cancer patients with bacteria (now known to be *Streptococcus pyogenes*).

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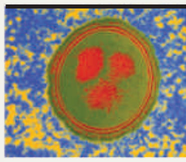
1909 Biologist Paul Ehrlich suggests that immune cells actively patrol the body to suppress the growth of cancerous cells.

DR MARK J. WINTER/ SCIENCE PHOTO LIBRARY

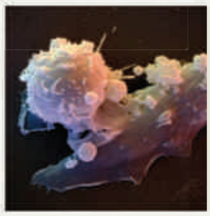
1953 Mice show immunity to tumour cells after cancer is allowed to grow and then surgically removed, suggesting the existence of tumour-specific antigens.

1957 Discovery of interferon, an immune-stimulating cytokine that will eventually be used as a non-specific cancer immunotherapy.



1959 Bacillus Calmette-Guérin (BCG), a tuberculosis vaccine, is shown to inhibit tumour growth in mice.

DAVID SCHARF/SCIENCE PHOTO LIBRARY



1973 Dendritic cells described by Ralph Steinman and Zanvil A. Cohn. Steinman won the Nobel prize for this discovery.

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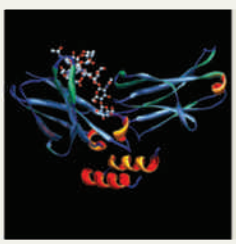
1983 Discovery of T-cell antigen receptors, which detect antigens presented by other immune cells and ramp up the immune response.

1986 First humanized antibodies approved by the US Food and Drug Administration (FDA).



1997 The first monoclonal antibody for cancer, Rituximab, approved by the FDA for treating non-Hodgkin's lymphoma.

DR MARK J. WINTER/ SCIENCE PHOTO LIBRARY

2008 First therapeutic cancer vaccine, Oncophage, wins approval in Russia for treating kidney cancer.

2010 FDA approves Dendreon's cancer vaccine, Provenge (sipuleucil-T), for the treatment of prostate cancer.

CANCER RESEARCH INSTITUTE

William Coley (centre) was the first to practise cancer immunotherapy a century ago.

BACTERIOLOGY

A caring culture

William Coley found a way to prompt the immune system to fight cancer over a century ago. After years of neglect, scientists are now seeking to replicate his success.

BY SARAH DEWEERDT

One day in October 2005, while working on what he hoped would become a widely effective cancer therapy, MacAdam went down to the Yale University archives to pore over hundred-year-old patient records, taking notes by hand. Back in the lab, his colleagues had tracked down an old-fashioned strain of bacteria, isolated from a patient who died of scarlet fever in 1924, and were experimenting with culture techniques found in dusty bacteriology textbooks — even growing the bugs in ground beef, a common approach in the nineteenth century.

MacAdam, chief executive of MBVax Bioscience, based in Vancouver, Canada, was attempting to replicate the success of a cancer vaccine developed at the end of the nineteenth century by a young doctor named William Coley.

Coley turned to bacteria to save his patients' lives. In 1890, wracked with regret over the death of his first patient from a soft-tissue cancer called a sarcoma, he searched the medical literature for anything that might have saved her. He read about another sarcoma patient whose tumour had mysteriously disappeared after a bacterial skin infection. Coley tracked the man down and found that he remained cancer free seven years after the infection.

The case was not unique. Coley soon found documented examples going back hundreds of years in which others had experienced such 'spontaneous regression' of cancer after infection. Reasoning that the infections could have prompted these patients' immune systems to fight their tumours, he turned to the bacteria that cured the man he had met. He deliberately infected one of his own sarcoma patients with *Streptococcus pyogenes*, and within weeks his patient made a dramatic recovery.

Coley continued to administer his therapeutic cancer vaccine to patients over the next four decades, tinkering with it along the way. He began using heat-killed bacteria to make the treatment safer, and added a second species to improve its effectiveness. He treated hundreds of people, curing more than a quarter of his sarcoma patients plus some with other types of cancer.

Even by today's standards, his results are remarkable. In 1999, researchers compared 128 of Coley's cases with 1,675 matched controls treated with modern cancer therapies, and found that his patients survived a median of 8.9 years, compared with 7.0 years for contemporary patients. Half of Coley's sarcoma patients lived for ten years, compared with 38% of those treated with modern therapy. Coley also improved the ten-year survival rates for patients with kidney and ovarian cancers. "What Coley did for his [sarcoma] patients back then was better than what we're doing for these same patients today," says Charlie Starnes, a researcher at Amgen, a biotechnology company based in Thousand Oaks, California, who has studied the history and mechanisms of Coley's therapy.

But after more than a century, the field of immunotherapy that Coley launched still hasn't come to fruition. Radiation and chemotherapy became established therapies in the mid-1900s, pushing Coley's vaccine out of favour. Chemotherapy and radiation were comparatively easy to standardize, while Coley's approach required careful calibration for each patient and didn't seem to work as well for other cancers as it did for sarcoma. Scientists didn't understand the underlying mechanisms, and some doctors couldn't replicate the results at all.

That may finally be changing, thanks to a better understanding of how the immune system functions, of the molecular mechanisms underlying infection and cancer regression, and of previously ignored details of Coley's work.

RECONSTRUCTING SUCCESS

Over the course of Coley's career he worked with many different bacteriologists who made more than 20 versions of the vaccine; some were more effective than others. "The vaccine that was made by a bacteriologist named Martha Tracy was clearly the most successful version," says Stephen Hoption Cann, chief medical officer at MBVax.

So the MBVax team set about trying to replicate Tracy's vaccine using modern laboratory techniques — and sterile culture media rather than ground beef. The reconstructed vaccine contains killed *S. pyogenes*, a spherical bacterium that commonly infects the throat and skin, and *Serratia marcescens*, a bright red, rod-shaped bacterium that contains an immune-stimulating pigment known as prodigiosin.

MBVax hasn't yet conducted controlled trials of its vaccine. But from 2007 to 2012, the company gave it to about 70 people with late-stage cancers, including melanoma, lymphoma and malignancies in the breast, prostate and ovaries. Tumours shrank in about 70% of the patients, and 20% went into complete remission, according to MBVax.

Several other groups are researching similar mixed-bacteria vaccines. In 2012, researchers in Germany tested a combination of heat-killed *S. pyogenes* and *S. marcescens* bacteria in a phase I safety trial in 12 cancer patients, and found that the vaccine increased levels of cytokines that enhance immune response. And even though it wasn't the aim of their study, they also documented tumour

regression in one participant.

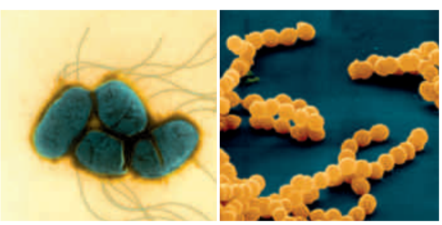
Not all patients respond, however, and MBVax wants to know why. But before it can do a clinical trial to answer those questions, the company must build a multimillion-dollar production facility that meets US and European standards for manufacturing pharmaceuticals. For now, MBVax has stopped vaccine production, but Hoption Cann says the company is seeking funding for the facility and hopes to begin development within two years.

Such difficulties are typical of the regulatory hurdles that any revival of Coley's therapy is likely to face. "In drug development, it's very difficult to get approval for a bacterial extract," says Uwe Hobohm, a biologist and bioinformaticist at the University of Applied Sciences in Giessen, Germany, who champions Coley's work. Instead, regulatory agencies prefer to see therapies based on individual molecules with well-defined mechanisms of action, rather than bacteria-based products that can include multiple active and inactive molecules and a variety of mechanisms.

MOLECULES AND MECHANISMS

Hobohm says that a more feasible approach might be to determine the mechanisms by which mixed-bacterial vaccines work, and to develop therapies based on specific molecules produced by bacteria but purified and standardized to make them acceptable to the US Food and Drug Administration (FDA). He believes Coley's success can be traced to a class of molecules known as pattern recognition receptor (PRR) ligands, which lend themselves well to this kind of regulated production.

Pathogens produce a variety of PRRs, including lipopolysaccharides, certain proteins and DNA. These molecules activate dendritic cells — a type of immune cell that recognizes pathogens and coordinates the initial stages of the immune response. In the past, many researchers thought the immune system did not attack cancer because it didn't recognize malignant cells as foreign. But Hobohm thinks that is only half the story, as a dendritic cell must encounter PRR ligands before it can fully activate T cells, in turn enabling them to recognize and destroy cancer cells. But because cancer cells don't make PRR ligands, the dendritic cells can't generate a robust response. "Usually the immune reaction is there, but it's not strong enough," Hobohm says. "We believe that the proper activation by dendritic cells is missing because PRR ligands are missing."



FROM LEFT: KWANGSHIN KIM/SCIENCE PHOTO LIBRARY; EYE OF SCIENCE/SCIENCE PHOTO LIBRARY

Serratia marcescens **(left) and** ***Streptococcus pyogenes*** **infections can have anticancer effects.**

DON MACADAM

An old bacterial vaccine next to its contemporary version.

Hobohm tested this theory by administering commercially available PRR ligands, which are purified from bacteria, to mice with experimentally induced tumours. Other researchers had previously tested only single PRR ligands, or multiple PRR ligands in combination but with only a few doses. By contrast, Coley administered his vaccine at least once or twice a week for several weeks or even months. Following Coley's lead, Hobohm gave his animals injections of three PRR ligands ten times over the course of three weeks. The approach cured four out of the five mice in the study.

One of the molecules used in the study, mistletoe lectin, is widely used as an add-on cancer therapy in Europe but was only recently shown to be a PRR ligand. Hobohm found that its structure is strikingly similar to that of a toxin produced by the bacterium *Shigella dysenteriae*, implying that, like the toxin, it probably triggers an immune response. Similarly, imiquimod, which is used to treat skin cancer, is thought to be a PRR ligand because of the way it interacts with immune cells. Hobohm believes that it might be possible to increase the efficacy of both therapies by combining them with other PRR ligands.

One barrier preventing further investigation of PRR therapy, however, is that the molecules often induce a fever — much like the bacteria from which they originate. And because fever usually indicates infection, it is classified as a negative side effect in drug development studies. Indeed, early versions of Coley's therapy, which included live bacteria, sometimes killed his patients. But those patients who had high fevers after receiving Coley's vaccine had better survival rates than those who experienced little or no fever.

Hoption Cann says unpublished data show that patients treated with the MBVax vaccine are more resistant to infection than other cancer patients, despite frequent treatment-induced fevers, which suggests that the fever is not harmful. Yet the prejudice against fever has made it hard for some researchers to get their studies funded, he adds.

SITE INSIGHT

Others doubt that fever is necessary, however. "Is temperature critical, or is it a bystander?" asks Simon Sutcliffe, chief medical officer of Qu Biologics in Vancouver, Canada. His company is taking a different Coley-inspired approach, and has seen promising results in patients with advanced cancer without causing a fever.

After reviewing Coley's data and spotting a pattern others hadn't detected before, Hal Gunn, the chief executive of Qu Biologics, began developing a type of therapy he calls site-specific immunomodulators (SSIs). Different species of bacteria infect different parts of the body, and Gunn noticed that Coley's therapy was particularly effective against cancers located in tissues that are most vulnerable to infection with *S. pyogenes* — the original bacteria Coley's research had targeted. "It came to me that it might be stimulating an immune response that was site specific," Gunn recalls. Qu Biologics has since developed a suite of vaccines, each derived from a single, organ-specific bacterial species: *Escherichia coli* for the bowel, *Klebsiella pneumoniae* for the lung, and so on.

Gunn and his colleagues speculate that their SSIs reset the immune system by mimicking the beneficial effects of infection at a tumour site. In particular, they believe that SSI molecules alter the activity of macrophages, a type of cell involved in the early stages of an immune response. The SSIs, they say, cause macrophages to shift from a response involved in tissue repair and cancer growth to a response that promotes the destruction of abnormal cells.

More than 250 patients with advanced cancer have been treated with SSIs from Qu Biologics. No randomized study of the therapy has been done, but an analysis by the non-profit Reliable Cancer Therapies found that SSI therapy increased median survival by 20 months among patients with advanced breast cancer, and by 12 months among those with a variety of late-stage cancers. Qu Biologics plans to begin clinical trials in late-stage lung-cancer patients in 2014.

Such studies fit into the larger vision held by advocates of Coley's work, who believe it is time to put historical research and inspiration to the test. "Clinical trials need to be carried out to show that this vaccine is beneficial for cancer patients," says Hoption Cann. "Until that time, interest in Coley's work comes and goes, but nothing stays around until you can demonstrate that." ■

Sarah DeWeerdt *is a freelance science writer based in Seattle, Washington.*

NEIL WEBB

DRUG DEVELOPMENT

Releasing the brakes

Tumours can put a brake on the immune system, but new therapies work by removing these brakes. Now, researchers have to figure out how to use them most effectively.

BY KAREN WEINTRAUB

First it was one melanoma patient, a woman named Sharon, who should have died but didn't. Then, several more outlived their prognoses — not just surviving but seeing their tumours shrink dramatically or even disappear. As the successes accumulated, in both individual patients and larger clinical trials, oncologist Antoni Ribas slowly began to accept that the immune treatments he was giving to his cancer patients were making a profound difference. Initially only about one in ten patients improved, but that fraction increased as he and his colleagues tested newer versions of the therapy. Ribas, a tumour immunology researcher, now has dozens of patients, like Sharon, whom he had expected to succumb cancer years ago. His patient load at the Jonsson Comprehensive Cancer Center at the University of California, Los Angeles (UCLA) used to stay about the same from one year to the next, with new melanoma patients roughly equaling the number who didn't make it. Now, the number of patients is growing.

The drugs he uses are known as immune checkpoint blockades and they are designed to circumvent one of the insidious ways in which cancer staves off an immune response. The immune system has a number of checkpoints — mechanisms that help to prevent it from getting out of control and attacking the body's own cells. The checkpoints act much like the brakes on a car: even if the immune system is trying to prompt its T cells into action, the checkpoints suppress the activation. Tumours can turn on these checkpoints and prevent a T-cell attack, but immune checkpoint blockades take the brakes off the T cells, freeing them to fight the malignancy.

When other researchers saw the results of clinical trials of checkpoint blockades in melanoma, they dismissed them as too narrow to be of much use in other cancers. Melanoma was different, they said, and has a known immune component. Then, in 2012, everything changed. In one study, a checkpoint blockade caused a measurable improvement in 31% of renal cancer patients, and in 18% of patients with lung cancer, which kills more people every year than colon, breast and pancreatic cancers combined[1]. Researchers and drug companies realized that these blockades, also called checkpoint inhibitors, might be as effective in patients with any type of solid tumour as they were in those with melanoma. Jedd D. Wolchok, a medical oncologist at Memorial Sloan-Kettering Cancer Center in New York City, says the lung

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cancer findings were "a pivot point for the entire field," changing immunotherapy from a niche, experimental approach into something that could eventually be considered a conventional cancer treatment. In 2013, larger studies confirmed the lung cancer results as well as showing similar benefits in patients with prostate, breast, kidney, colon and other cancers.

The findings have been so tantalizing that researchers started asking a simple question: if one checkpoint blockade drug could do so much for a small proportion of patients, could a cocktail of several such drugs — or a combination of checkpoint blockades with chemotherapy, genetic treatments and other types of immune therapies — help more of them? "It's not realistic to think of immunotherapies only as single agents," says Lawrence Fong, a cancer immunologist and haematological oncologist at the University of California, San Francisco. "Combinations will probably be needed to realize the full potential of cancer immunotherapy."

SURPRISING HUMAN STUDIES

For more than half a century, scientists have been trying to turn the body's immune system against cancer. But decades of failures have revealed that tumours have the ability to evade, tamp down and overwhelm the normal immune response. Most modern immune therapies try to get the immune system to recognize and attack tumour cells (see 'Honing that killer instinct, page S13). One such treatment, the vaccine sipuleucel-T (marketed as Provenge by Dendreon Corporation in Seattle), was approved by the US Food and Drug Administration in 2010 for use in prostate cancer — a move that generated a lot of excitement. But the drug has proven disappointing, with benefits limited to a small percentage of patients; Dendreon is now reported to be for sale.

The problem, researchers have slowly been realizing, is that stepping on the immune system's gas pedal isn't enough: it is also necessary to release its brakes — and that's where immune checkpoint blockades come in. Eighteen years ago, James Allison, now an immunologist at the MD Anderson Cancer Center in Houston, Texas, figured out how to do just that. Allison, then at University of California, Berkeley, noticed that one checkpoint protein, called CTLA-4 (cytotoxic T-lymphocyte-associated antigen 4), seemed to prevent T cells from attacking tumours. So he blocked CTLA-4 activity in mouse models of various cancers (including melanoma) and, to his surprise, some of the mice experienced complete remission.

In 2011, the US FDA approved the anti-CTLA4 drug ipilimumab (developed by Bristol-Myers Squibb and marketed as Yervoy), which was based on Allison's research and eventually saved the lives of some of Ribas's patients. Allison says the reality has been even better than he expected. Mouse studies suggested that ipilimumab wouldn't work well by itself and would need to be combined with other drugs to show any significant effect. But the first patients responded to ipilimumab even better than the mice had.

CTLA-4 isn't the only checkpoint being targeted by researchers and drug developers. Early trials suggest that drugs that block a different checkpoint, called PD-1, are even more effective and have fewer side effects than ipilimumab[2]. In recent studies, checkpoint blockades produced improvements in between 20% and 65% of patients, depending on the drug, dosage and type of cancer. In one long-term study of ipilimumab in patients with advanced melanoma, 22% of the 1,861 patients survived for three years, and 17% for seven years or longer (with median survival nearly a year); historically, average survival was six to nine months[3]. Early research suggests that ipilimumab may be even more effective when combined with other drugs.

"Checkpoint blockades take the brakes off the T cells, freeing them to fight the malignancy"

In further evidence for the value of drug combinations, ipilimumab and nivolumab (another Bristol-Myers Squibb drug, which targets PD-1) appear to complement each other. In one study published in early 2013, 53% of patients with melanoma who took the highest safe doses of both drugs showed reductions in tumour size of 80% or more[4]. But not everyone fared well. Nearly one-fifth of the subjects involved in the study suffered severe, though treatable, side effects, including pancreas and liver dysfunction, itchy skin, lung inflammation, and uveitis (inflammation of the eye).

Drug companies consider these side effects manageable, and remain enthusiastic about checkpoint blockades. Merck, for instance, is testing its PD-1 blockade, MK-3475 (also called lambrolizumab), in seven clinical trials that are expected to enroll more than 3,000 patients with bladder, colorectal, head and neck, melanoma, non-small cell lung and triple-negative breast cancer (so-called because it doesn't express three of the most common genes). Although most of that research is being done using MK-3475 alone, "we are especially interested in combinations with other immunomodulatory agents," says Eric Rubin, vice-president of oncology clinical research at Merck, which is based in Whitehouse Station, New Jersey. The company is testing MK-3475 in combination with several chemotherapy drugs, including carboplatin, cisplatin and pemetrexed.

Bristol-Myers Squibb is also pursuing combinations of checkpoint blockades with other therapeutic approaches. One combination the company is testing pairs ipilimumab with the cancer vaccine sipuleucel-T. Tests in mice suggest that this mix should work well, says Glenn Dranoff, an oncologist at Harvard's Dana-Farber Cancer Center who helped to develop the vaccine. And, following a successful phase I trial[4], Bristol-Myers Squibb is pursuing phase II and III trials of ipilimumab combined with nivolumab in patients with melanoma. But they're also hedging their bets. "We have to be prepared for the possibility that this is not the optimal combination," says Nils Lonberg, senior vice-president of biologics discovery at Bristol-Myers Squibb.

Lonberg points to the fact that, among other combinations, Bristol-Myers Squibb is testing a drug called lirilumab together with ipilimumab and with nivolumab, in separate phase I trials involving patients with various types of cancer. Lirilumab, a human monoclonal antibody, promotes activation of a different part of the immune system — the natural killer cells — to attack the tumour. The goal is to fight the disease with both arms of the immune system simultaneously — the innate, nonspecific natural killer cells and adaptive T cells, which are finely adapted to respond to new insults. "There is the possibility of synergy between overcoming blocks to the innate anti-cancer response and overcoming blockade of the adaptive immune response to cancer," he said. "We're looking very closely to see whether or not that occurs."

HUNTING FOR THE BEST MIX

Ultimately, the right treatment combination is going to depend on many factors — the type of cancer a patient has, as well as their genetics, age, race and gender. Figuring out which combinations will work best for which patients is going take years of trial-and-error experimentation, and is likely to be risky for both drug companies and cancer patients. For instance, some chemotherapies could end up suppressing the immune system instead of supporting it, warns Keith Flaherty, an oncologist at Massachusetts General Hospital and Harvard Medical School in Boston who specialises in melanoma. "There's the concern that immunotherapy and chemotherapy would in fact be antagonistic," he says. But Flaherty is optimistic about the potential intersection of checkpoint blockades with therapies that target the specific gene mutations that are found in various tumours — such as the *BRAF* mutation that is common in melanoma patients. "If you want a drug that specifically counters some of the mechanisms by which tumours escape immune surveillance, targeted therapies are the place to look."

Flaherty criticizes the haphazard nature of much of today's immunotherapy research. Some pharmaceutical companies, he says, are trying combinations without understanding the biology behind them. "That's pretty unscientific." In addition to putting cancer patients

REUTERS/DAVID MCNEW

Cancer patient Stew Scannell receives intravenous Lambrolizumab during a clinical trial at UCLA.

at risk, such a scattershot approach is more likely than rationally designed approaches to fail, which could pull down the whole immunotherapy field.

Flaherty also has an even more basic concern: with so few biomarkers available to identify which patients are most likely to respond to checkpoint blockades and other immune treatments, is it even possible to study combination therapies? "I worry that we don't have a scientific and rational way to develop combination immunotherapy," he said. "That doesn't mean I don't think it should be pursued, but a steady focus needs to remain on the mechanism of interaction between different classes of therapies."

Researchers are also pushing for a better understanding of why the successes of immune blockades are so uneven. Why don't all patients respond to them in the same way? "That's a very important question to answer," says Rafi Ahmed, an immunologist and director of the Vaccine Center at the Emory University School of Medicine in Atlanta, Georgia. "It might give us insights into combination therapies and also perhaps allow us to target additional approaches."

If biomarkers can be identified, they could help scientists and clinicians to pair the appropriate immune therapy or combination with the right patient. Early research, for instance, suggests that people whose tumours express the molecule PD-L1 are more likely to respond to PD-1 blockade treatments than those whose tumours don't express PD-L1, says Suzanne Topalian, director of the melanoma programme at Johns Hopkins University's Sidney Kimmel Comprehensive Cancer Center in Baltimore, Maryland. The association between protein expression and drug effect is logical, given that the presence of PD-L1 suggests that these patients have active blockades of precisely the kind that these drugs target.

Despite their checkered successes, checkpoint blockades are proving exciting enough, and generating enough promising data, that in April 2013, the FDA labelled Merck's MK-3475 a "breakthrough therapy" in recognition of the dramatic clinical effect seen in a phase I melanoma trial. Designation as a breakthrough therapy is meant to speed up the development and review of a candidate drug that shows promise for treating a life-threatening disease. The FDA is working with investigators and pharmaceutical companies to accelerate the development of these drugs, Topalian says.

> ***"Patients treated with immune therapies could potentially gain a lifetime of protection."***

SHIFTING PERSPECTIVES

For decades, scientists have focused on cancer genetics and on designing treatments that counteract specific mutations. But now they need to broaden their focus, says Ira Mellman, vice-president of research oncology at Genentech, a member of the Roche Group that is based in San Francisco, California. Checkpoint blockades and other immune therapies don't work on the same genetic model of cancer that has dominated research and treatment for more than forty years. "We now know that genes are not the whole story, because we have all sorts of drugs targeted to oncogenes and people don't get cured," Mellman says. The success of checkpoint blockades is slowly driving researchers away from the genetic view of cancer — and without this necessary shift in perspective, Mellman says, progress will stall and immune therapies won't reach their potential.

Doctors must also adapt their clinical strategies so that they can effectively use checkpoint blockades and other immune-based approaches. Patients treated with immune therapies often show different patterns of response from those treated with standard drugs, Topalian and others say. With chemotherapy and genetic approaches, success is typically measured by a decrease in tumour size, and if a patient is going to improve it usually happens relatively quickly. With immune therapies, however, it's not unusual for it to take several months before the cancer begins to visibly recede. Sometimes, tumours can even get larger at first as T cells and other immune cells flood to the site. "Physicians using these drugs really need to be well-educated about response patterns," Topalian says. "With these drugs, the response may not occur until later on, and then you have to make a decision about whether to continue treating the patient."

Right now, it's unclear how long patients will need to be on immune-blockade treatments. Ribas is using MK-3475 for many of his patients, and is giving them infusions every two to three weeks. He plans to keep each patient on the drug for two years and then pause the infusions to track how the patients respond. "There's not enough data to say when we can stop or whether we need to continue," he says. Ideally, a patient's immune system would eventually be able to take over and eliminate the cancer, or at least keep it in check indefinitely.

Topalian notes that patients treated with immune therapies could potentially gain a lifetime of protection, similar to the buffer against certain diseases offered by childhood vaccines. "We hope that the same thing is happening in cancer," she says. "We hope that we are re-educating the immune system and that, even if it doesn't completely destroy every last cancer cell, it can keep it in check for a very long time." ■

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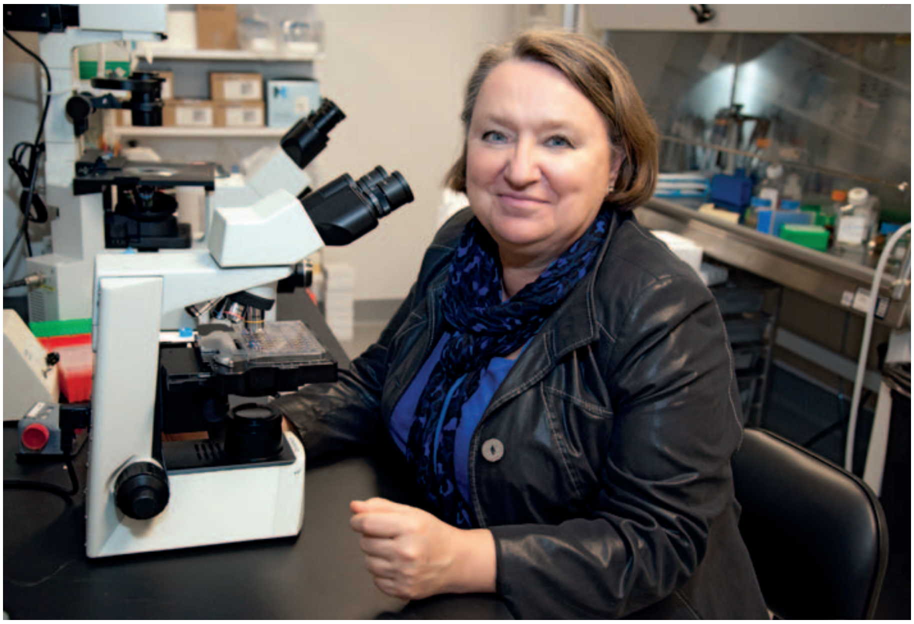


BAYLOR RESEARCH INSTITUTE, DALLAS, TEXAS

Q&A Karolina Palucka

Evidence presenter

Immunologist Karolina Palucka, at Baylor Institute for Immunology Research in Dallas, Texas, helped treat Nobel prizewinner Ralph Steinman's pancreatic cancer with dendritic cells — the cells he co-discovered. Here she explains the use of dendritic cells in cancer immunotherapy.

What are dendritic cells, and how do they work with T cells to create a cancer vaccine?
Immunity results from a complex interplay between the adaptive immune system (which is antigen-specific) and the innate immune system (which isn't). B cells and T cells of the adaptive immune system use receptors that recognize antigens, or their derived peptides, in a highly specific manner. Dendritic cells (DCs) provide an essential link between the innate and adaptive immune responses.

The generation of anticancer immunity depends on DCs presenting cancer antigens to T cells. But cancers can create an environment that inhibits T cells. The aim of DC vaccination is to boost cancer-specific effector T cells that can not only fight existing cancer but also induce immunological memory to control the recurrence of cancer.

What approach did you and Ralph Steinman take to treat his pancreatic cancer?
It was a cell-based therapy. In this approach, you take DC-precursor cells from the blood of a cancer patient, differentiate and activate them in culture, load them with tumour antigens, and then inject the cells back into the patient. The hope is that these DCs will activate tumour-specific T cells. The tumour antigens can either be shared antigens, which are expressed by many cancers, or patient-specific antigens. With Ralph, it was patient-specific: together with colleagues from other institutes, we studied cells from his tumour to work out which antigenic sequences to go after.

What was the outcome?
Ralph received eight injections of this vaccine over the course of eight months, in combination with a chemotherapeutic drug called gemcitabine. After each injection, we saw an expansion of T cells specific to the vaccine antigens in his blood, so it was clear that the DC treatment boosted the immune response against the cancer. Although we can't say for sure that this treatment was responsible, Ralph survived for 4.5 years after his diagnosis — something that only around 5% of patients with this disease achieve.

How are you following up on this approach?
We wanted to see if this could help other patients with pancreatic cancer. We've developed a full clinical trial that we're hoping will start in early 2014, in which we'll try this approach in a larger number of patients with the same diagnosis.

Will this trial be as personalized?
No. To make it more feasible we're combining two approaches. We're using a shared tumour antigen called mesothelin. But there are shortcomings to shared antigens. Not all patients will express them, and some shared antigens are also expressed by healthy cells. That's why, in patients from whom we can get tumour samples, we're going to try to identify patient-specific mutations and go after these in the boosting phase of the trial. I think a combined approach like this represents a more realistic view of what we can do in the clinic, and will be the way things are going to go.

What roles do you see emerging for DC vaccines?
The cancer immunotherapy field has made tremendous progress, thanks to the development of antibodies against immune-suppressing molecules, such as PD-1 and CTLA-4, that are expressed by cancer cells. We're seeing promising results of clinical trials with these antibodies, but they still don't work for all patients.

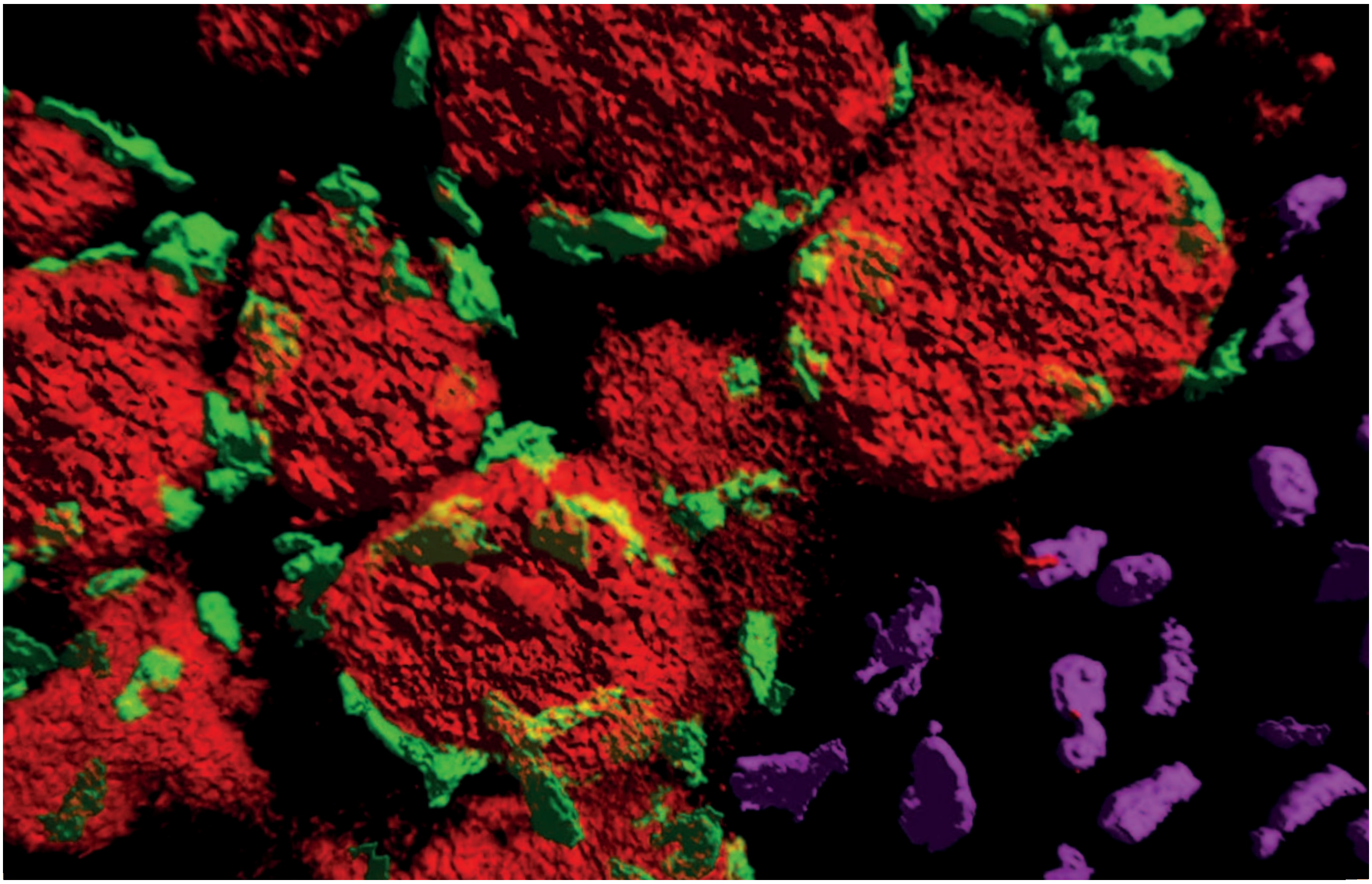
One reason could be that if there isn't a pre-existing immune response against the cancer, blocking these molecules may not be enough. This is one area in which DC vaccines, because of their ability to prime immune responses, could make a big difference. So far, however, we have seen a discrepancy between the immunogenicity of DC vaccines and their clinical efficacy — for example, some patients who get an immune boost in response to the vaccine nevertheless show no tumour regression. I think we can diminish this discrepancy by combining DC vaccines with immunomodulatory antibodies.

Another potentially big area is using DCs for preventive vaccination of people with cancer mutations, such as *BRCA1*, to prevent progression from premalignant to malignant disease.

How can we develop better DC vaccines?
The various types of DCs interact differently with the immune system. So one exciting area is exploring how subsets of DCs contribute to human immunity, and how they each respond to inhibitory factors. We also need to enhance platforms where academia and pharmaceutical companies can collaborate to test therapies at an early stage of their development and do more mechanistic studies, so we can better understand what the vaccines are doing.

I have been in this field for 17 years and this is the best time for our research. We have made tremendous progress and there is a lot of optimism. ■

INTERVIEW BY MARIAN TURNER



MATTHEW KRUMMEL AND JOHN ENGELHARDT

Two types of immune cells (green and purple) near the border of a tumour (red) are caught in action in this video still.

MEDICAL IMAGING

Removing the blindfold

Using a variety of creative imaging techniques, researchers are tracking the dynamic interactions of immune and cancer cells. Their results will guide drug development.

BY KATHERINE BOURZAC

Mark Headley's computer screen displays the cellular landscape of a living mouse lung, software-corrected to ensure the images do not blur with each rapid breath. Headley, a post-doc in immunology at the University of California, San Francisco (UCSF), points to certain features: the spherical black areas are alveoli, air pockets absent of cells; the blue threads are fluorescently labelled structural proteins within cells; and the reddish tubes are groups of labelled platelets flowing through blood vessels. So far, so good.

Then, like something out of a Hollywood B movie, a neon green blob enters the scene: a cancer cell. The blob stretches out. "It looks like it's trying to escape the blood vessel," says immunologist Matthew Krummel, Headley's boss. Green fragments break off the blob. Krummel doesn't yet know what is happening on screen. The cancer cell may be dying, it may be sending out signals to the immune system, or it may be doing something else entirely.

Krummel and other immunologists are making such videos to get a better grasp of the immune system's response to cancer. Trying to understand immunity with conventional static images is like trying to figure out the game of football by looking at a photograph of players on the field, says Thorsten Mempel, a doctor and immunologist at the Massachusetts General Hospital in Boston. By imaging at the cellular and molecular levels, researchers are starting to learn the plays. They can see not just how much a tumour grew or shrank, but the details of what happened and why.

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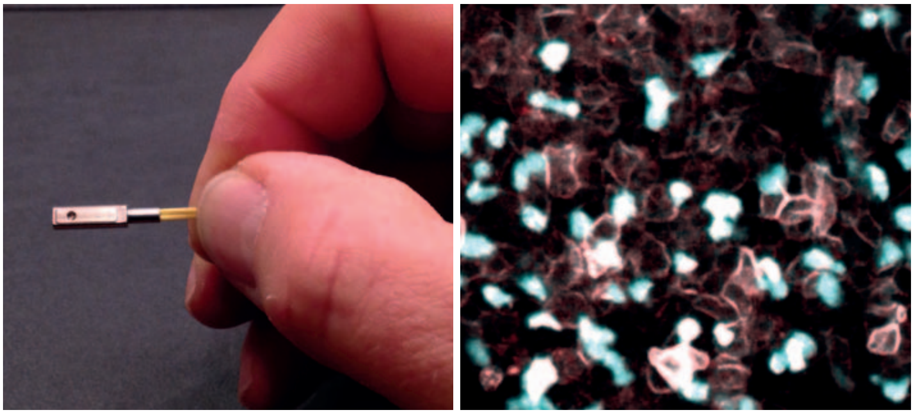
Cancer immunotherapy comes of age:
go.nature.com/xocvhw

Initially, treatments that recruit a patient's own immune cells to combat cancer were developed without the kinds of studies Krummel and others can now perform. Researchers couldn't see what the cells actually did in the body, so they were unable to spot problems, says Christopher Contag, an immunologist at Stanford University in Palo Alto, California. "We're seeing the failure of a lot of immunotherapies because we went in blindly," he says.

By watching immune reactions to cancer in detail, and turning a lens on what happens during experimental immunotherapies, researchers hope to take the blindfold off.

CELLULAR GPS

Over the past ten years or so, scientists have been watching fluorescently labelled immune and tumour cells in living animals using sophisticated microscopy technology. They have learned that *in-vitro* experiments, which are often the first step in the development of immunotherapies, can be very misleading.



An implantable microscope (left) is being developed to capture immune-tumour cell action (right).

LEFT: CHRISTOPHER CONTAG/ RIGHT: BÉATRICE BREART AND PHILIPPE BOUSSO, INSTITUT PASTEUR

Many of the things that cells do in culture, they don't do in the body. "Every time we set up a new disease model with imaging we find something unexpected," says Contag.

One early finding is that immune cells inside the body take their time. Biologists are using fluorescent labels to track cells and figure out where they are going, how long it takes them, and what other cells they interact with. Immune cells inside the body interact with one another and with cancer cells for much longer than they do outside the body. In cell culture, for example, an immune cell called a cytotoxic T cell will kill a cancer cell in a matter of minutes. In a mouse, however, it's a different story. According to work by Philippe Bousso, an immunologist at the Pasteur Institute in Paris, in the body it takes an average of six hours for a T cell to kill a single cancer cell[1].

Bousso was one of the first to look at tumour–immune cell interactions in living animals using multiphoton microscopy, which allows researchers to view cells as deep as 400 micrometres beneath the skin. Conventional microscopes, which use single photons of visible light to excite fluorescent particles, can peer only about 50 micrometres deep. The infrared light used for multiphoton microscopy is of a lower energy than visible light, so the fluorescent dyes must absorb multiple photons in order to get excited and shine; but infrared light also travels deeper into tissue without scattering. In living mice, 400 micrometres is deep enough to view breast, prostate and skin-cancer tumours.

This multiphoton microscopy enabled Bousso to take a new look at adoptive-cell therapy (see 'Honing that killer instinct', page S13), a treatment in which killer T cells are taken out of the body, trained to target cancer cells, then multiplied and reinjected. His results suggest that one reason these therapies don't work well for most cancers is because each T cell takes more time to kill a single cancer cell than expected, and solid tumours are made up of a massive number of cells. For these therapies to work, the dose of T cells might need to be much greater than what's been tested to date.

The broader implication, however, is that cancer researchers might be wise to re-evaluate their approach. Cancer immunology studies conducted *in vitro* are not always realistic, and conclusions about cell behaviour should be tested in animal models before being accepted and used as the basis for new therapies. "What's going on *in vivo* is difficult to access," says Bousso. The only way researchers can know for sure is to observe their subjects.

Such studies have found immune cells dilly-dallying inside the body. Krummel, for example, recently published research showing killer T cells located at the periphery of breast cancer tumours in mice, interacting with other immune cells called dendritic cells, but never entering the tumour[2]. It was just one more piece of evidence that, in the battle between the immune system and cancer cells, there are many other factors that must be better understood and considered in designing new immunotherapies.

CHEMICAL EAVESDROPPING

To get a better understanding of these complex interactions, researchers are now moving beyond simply tracking cells' locations. "We're trying to get a more detailed picture, at the subcellular and molecular level," says Wolfgang Weninger, a cell biologist at the University of Sydney in Australia. "We don't understand what T cells do when they're put back into the body."

Capturing these details presents imaging researchers with major technical challenges. There are only so many colours of fluorescent proteins, therefore parsing the signal becomes problematic — a few years ago, imaging studies were limited to just four labels at a time. To see more, Krummel's group at UCSF has built its own microscope with two US$150,000 infrared lasers that fire at intervals of 1/30th of a second, each at a different wavelength, to excite different imaging labels that are activated by different colours of light but emit similar colours. Using this method, the group can image six labels at once.

"We're seeing the failure of a lot of immunotherapies because we went in blindly."

Whereas Krummel's microscope relies on speedy frame rates, other researchers are able to get detailed information by snapping higher-resolution pictures. They're taking advantage of a technique called super-resolution microscopy, which makes it possible to image individual protein molecules *in vitro* both on the surface of and within cells. The technique appears to defy the laws of physics. That is because conventional lenses focus light on a spot with a minimum diameter of half the wavelength of light — a barrier called the diffraction limit; researchers, however, have devised ways to get around this constraint.

One early adopter of super-resolution microscopy in immunology is Daniel Davis, a biophysicist at the University of Manchester in the UK. Davis has built a microscope that uses not one but two lasers. One laser excites fluorescently labelled proteins in his sample, while the second laser creates a doughnut-shaped beam of light around the outer rim of the first, cancelling out the excitation of dyes in this region before they have a chance to shine. Typical confocal microscopy collects fluorescence from as small a field as 200 nanometres. But Davis's technique effectively narrows the focus of the light to a donut hole of just 10–20 nanometres, enabling the detection of single molecules (see 'Light show', page S12).

Davis recently used super-resolution microscopy to watch what happens when an immune cell called a natural killer (NK) cell attacks its target[3]. NK cells are part of the innate immune system, the first line of defence that targets cancer cells and foreign invaders non-specifically. NK cell's ability to kill abnormal-looking cells may be what prevents some people from getting cancer in the first place. These cells kill by delivering a membrane-bound payload of deadly proteins called a granule. But as the interior of NK cells is crowded with a thick mesh of structural proteins, Davis wondered how the granules passed through the mesh.

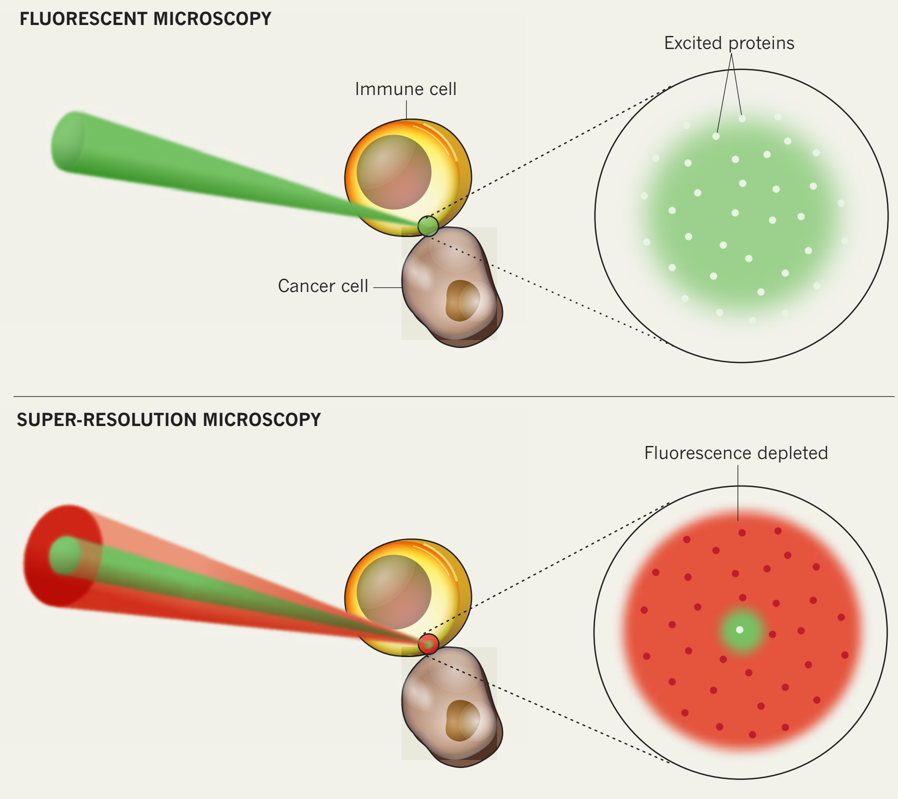
The super-resolution microscope provided the answer as Davis's group watched the process unfold. Directly beneath the cell membrane at the immune synapse, the protein structure that forms a bridge between a killer immune cell and its target, the structural proteins clear a pathway out of the cell for the granule. Although this new insight into cell biology is fairly basic, Davis believes these kinds of studies will lead to new drug targets.

BIG PICTURES

Davis reckons this is just the beginning. Eventually, he says, "we want to see where every single protein is on the surface of these cells." That's key to understanding the cell-level decisions that determine a cancer patient's prognosis. Immune cells receive multiple, often conflicting signals — some activating, some calming — that they must integrate before

LIGHT SHOW

Fluorescent microscopy uses a laser beam (green) to excite labelled proteins inside a cell so they shine, but it illuminates a field containing several molecules. In order to image a single molecule, a second laser beam (red) narrows the imaging field so that single proteins can be detected.



determining whether or not to kill a cell. The subtle details of these cellular actions, Davis says, are at the root of the variation among people both in their tendencies to develop cancer in the first place and in their response to treatment.

Although it is still early days for *in vivo* cell-imaging research, it has potential clinical implications. Imaging the immune system in detail can help researchers who are working on therapies to stay on the right track, says Stanford's Christopher Contag. Indeed, over the past decade, with the help of imaging, his group has been developing a novel immunotherapy both *in vitro* and in mice. They've recently applied to start a clinical trial.

Rather than using immune cells that recognize specific antigens associated with a patient's cancer, Contag's group is developing a therapy based on a class of innate immune cells called natural killer T (NKT) cells, which are distinct from the NK cells in Davis's study. The NKT cells are loaded up with tumour-killing viruses and are, says Contag, professional tumour-homing cells. They're experts at finding tumours, but slow at killing them. The viruses, on the other hand, do a poor job of locating the tumour — Contag refers to these viruses as "dumb particles" because they're not very good at finding their way around. But two days after the viruses reach the tumour, they multiply their numbers a million-fold and endow the NKT cells they inhabit with major tumour-killing prowess.

Loading innate immune-cell navigators with viruses might make for a killer combo, but it was only with the advent of better imaging that the possibility was taken seriously. In fact, researchers had dismissed the idea as a non-starter until Contag studied the dynamics of the system under the microscope.

> ***"Every time we set up a new disease model with imaging we find something unexpected."***

This lack of interest in the strategy made sense, given the previous state of knowledge about how NKT cells behave. It takes about 48 hours for the virus to incubate in the cell and emerge. But because mouse circulation is speedy, researchers assumed that once the virus-loaded NKT cells were injected back into the mouse they would reach the tumour in two hours — way too soon to do much good. And during these two days things could go wrong: interactions with other cells in the tumour's proximity, for example, might prevent the NKT cells reaching their target.

Imaging studies, however, dispelled these fears, revealing that viral release in mice is actually ideal. For whatever reason, the virus-infected cells reach peak accumulation within a mouse's tumour 48 hours after injection, not the expected two hours. In Contag's videos, all the tumour cells are infected simultaneously and bloom fluorescent green with labelled virus as it multiplies exponentially, eventually causing the tumour to collapse like a deflating balloon. And over time, the virus elevates the animals' immune response so that they're protected against recurrence.

Contag hopes to see the same response in late-stage ovarian cancer patients in his proposed clinical trial as he has in animals: not just temporary remission followed by a relapse, but long-lasting immune protection against the cancer. He believes that the immune response elevated by the viral infection can lead to the formation of memory immune cells that respond to and fight back against any tumour regrowth. The tumours tend not to grow back in mice; ideally, the same would happen in people.

The Stanford group is now working on an implantable microscope to do cell-level imaging of tumours deep inside the body and their response to immunotherapies. The device, which his group recently began testing in animals, is about the size of a fingernail and sends and receives light through optical fibres that snake in and out of the body. Its resolution, 0.1 micrometres, is half as good as that of a conventional microscope. However, Contag says that it avoids the need to anaesthetize the mice, cut them open and place them on a microscope objective and enables the researchers to take images over much longer periods.

Now that detailed, cell-level imaging has expanded immunologists' view of therapies in animal models, Contag wants to bring that capability to clinical trials, too. He is developing a non-invasive version of his microscope, which looks much like a laser pointer connected to a cable. A clinician could hold it against a patient's skin and use it to count labelled cells flowing past in blood vessels close to the skin. Such a device could provide a non-invasive way to spot circulating tumour cells — which signal that a tumour is coming back, or metastasizing, or both — in patients.

Detailed, dynamic imaging is enabling immunologists to better understand what really happens in that fast-moving game of immune-system football. Being able to watch the players in real time will lead to more insightful research and smarter drug development. "By understanding the rules," says Mempel, "we can change the game in our favour." ■

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PEGGY PETERSON PHOTOGRAPHY/PENN MEDICINE

T cells taken from a leukaemia patient and multiplied in culture are ready for infusion.

ADOPTIVE CELL THERAPY

Honing that killer instinct

Genetically altered immune cells are helping to push life-threatening cancers into remission and generating a buzz.

BY COURTNEY HUMPHRIES

A few years ago, when Michel Sadelain spoke about adoptive cell transfer (ACT) therapy at cancer meetings, his colleagues were dubious about what seemed a drastic and unconventional approach: harvesting and genetically altering his patient's immune cells to train them to attack her cancer. "I can't tell you how many nearly empty rooms I've spoken to about this technique," says Sadelain, director of the Center for Cell Engineering at Memorial Sloan-Kettering Cancer Center in New York.

The technique harnesses the power of the immune system by recruiting the body's own T cells — immune cells that recognize and marshal an attack against foreign invaders and diseased cells. T cells travel through the body, using their receptors to scan for small bits of protein called antigens on the surface of foreign cells. If an antigen matches the receptors, the T cell activates and launches an attack. In theory, malignant cells should be ideal targets for T cells, but tumours have ways of shielding themselves from an immune attack. With ACT, scientists tweak the T cells to give them a fighting chance. Sadelain calls them "living drugs".

Pilot studies in the past couple of years have had promising results, leading to increased interest and dozens more clinical trials investigating the technique. Success stories — albeit involving small numbers of patients — tell of people with aggressive cancers whose tumours melted away in days or weeks. In a field where extending life by a few weeks or months is considered a breakthrough, the complete remission of even a few patients is stunning. Sadelain is no longer speaking to empty rooms. Suddenly, he says, ACT has captured the imagination of scientists and pharmaceutical companies as if it were a new approach, rather than a field that has been developing for twenty years.

But there are both scientific and logistic challenges to expanding the use of this therapy. Researchers are still learning to control the cells' potency to ensure they can vanquish cancer without damaging normal tissue — an issue complicated by the fact that many cancer antigens are also found on normal cells. Another problem is that it's not yet clear how to turn ACT into a profitable business model, as harvesting and growing living cells requires much more time and skill than prescribing a drug. So while pharmaceutical companies are licensing proprietary receptors and looking into ways to scale up the process, that's just the start of the endeavour. As with any therapy, the companies still need to embark on large, multicentre clinical trials to test the effectiveness of the therapies on a broader group of patients. But large trials also require a way to engineer and distribute large quantities of cells, so they will only happen if companies are confident of long-term profitability.

Proponents of the approach say that the possibility of eradicating life-threatening tumours makes these challenges worth tackling. And recent progress in designing ACT therapies that are surprisingly effective is causing many in the field to sit up and take notice.

BOOSTING THE BODY'S CELLS

There are three strategies for ACT therapies (see 'Cellular attack'); the most-developed of which is the simplest. The tissue surrounding a tumour is likely to contain immune cells with antitumour activity, so doctors take a sample of this tissue and select those T cells that have been primed to attack the cancer. They culture these cells in the lab until they have enough, and re-infuse the cells back to patients along with the T-cell growth factor interleukin-2 (IL-2), which promotes the proliferation of antigen-specific T cells. However, the endogenous immune system has suppressive mechanisms that keep the immune response in check, and these mechanisms also prevent the newly transferred cells from working effectively. So patients must also be treated with drugs or radiation

to deplete their endogenous immune cells and allow the newly infused T cells to gain hold and fill the body.

This approach, called tumour-infiltrating lymphocyte (TIL) therapy, has been used successfully to treat only one type of cancer: metastatic melanoma. T cells that have been primed to attack a specific cancer are difficult to collect in a blood sample, but in melanoma these lymphocytes enter the tumour and are easy to biopsy. Over the past 25 years, a group led by Steven Rosenberg, an immunotherapy researcher and chief of surgery at the US National Cancer Institute in Bethesda, Maryland, has been building evidence that TIL therapy can alleviate or even eradicate melanoma in some patients.

"In the last of our trials, 40% of patients underwent complete, durable regressions of their melanoma," Rosenberg says of his latest results[1], published in 2011. Many of those patients had tumours throughout their bodies and had exhausted other treatments. This success vividly demonstrates how T cells sufficiently tuned to a specific cancer can have potent, long-lasting effects — and can even eradicate some tumours entirely.

But the current TIL regimen faces two big problems. First, patients must wait 4–6 weeks for the cells to grow before they can start therapy. The second problem is the need for specialized cell production facilities and staff trained in genetically modifying and growing the cells. Cassian Yee, a cancer immunologist at the University of Texas MD Anderson Cancer Center in Houston, says that work is being done to improve ACT and expand its use. New methods could make it possible to grow cells in days, rather than weeks, and research is underway to make it easier to obtain cells and to be more selective in the cells that are harvested. Yee and his colleagues have been developing methods to isolate tumour-specific cells circulating in the blood, for instance — a technique that could eventually make it feasible to treat cancers for which biopsies are hard to obtain or in which immune cells do not accumulate around the tumour.

KILLER CELLS

The success of TIL in melanoma is not currently transferable to other cancers, because it is harder to collect tumour-specific T cells. For those cancers, researchers are working to genetically modify T cells to hone their cancer killing skills. This strategy not only circumvents the need to find tumour-specific cells, but also allows scientists to tweak them in specific ways.

To do this, researchers are taking a couple of approaches. One option, called T-cell receptor (TCR) therapy, involves giving the cells new receptors that allow them to recognize specific cancer antigens; the receptors can even be modified to improve their ability to find and bind to their targets. To incorporate the new receptor, clinicians harvest a patient's T cells and then use a viral vector to deliver into the cells a gene that encodes the new receptor. The cells can also be engineered to express immune factors that prompt growth, that allow them to persist in the body, or that trigger other cells to attack the cancer. So far, TCR therapies have been shown to shrink tumours in some patients with metastatic melanoma, colorectal cancer and synovial sarcoma[2,3]. But there is one difficulty: the T-cell receptors must be genetically matched to the patient's immune type.

A more flexible tactic, called chimaeric antigen receptor (CAR) therapy, avoids this constraint. It uses a gene that encodes artificial, antibody-like proteins that bind the antigens studding the tumour cell's surface without needing to match the patient's immune type.

There are three pieces to CARs: an antibody that binds to a common cancer antigen; part of a receptor that activates the cell; and one or more stimulatory molecules that help the T cell proliferate and persist. When the CAR is inserted into and expressed in a T cell, it acts as a switch. As soon as the CAR encounters a matching antigen, it puts the T cells into attack mode. "The antibody provides the right conditions to find the tumour, and the T cell does the dirty work," explains Carl June, director of translational research at the University of Pennsylvania's Abramson Family Cancer Research Institute in Philadelphia.

Although conceived in the late 1980s, CAR therapies have only recently yielded positive results in small clinical trials. So far, they have all centred on the CD19 protein, which is expressed in B-cell leukaemias and lymphomas. CD19 is also expressed in normal B cells, which produce antibodies as part of the immune response. This means that CAR-initiated attacks can target healthy B cells, although the loss of these cells can be managed by therapies that treat antibody deficiencies.

In 2011, June and his colleagues reported that CAR T cells that target CD19 led to the remission of tumours in three patients with advanced chronic lymphocytic leukaemia (CLL) in whom multiple rounds of chemotherapy had failed[4]. Two of the patients experienced complete remission. In another study[5], led by Sadelain's group at the Memorial Sloan-Kettering Cancer Center, a different anti-CD19 CAR therapy led to remission for three of five adult patients with acute lymphoblastic leukaemia (ALL). Adult ALL is a terrible disease and the patients had already relapsed twice after chemotherapy, so these are "spectacular responses," Sadelain says. Researchers are now investigating whether CARs can be as effective against solid tumours as they are against blood cancers.

ON TARGET

Now that small clinical trials have shown that engineered T cells can effectively treat some forms of cancer, researchers must optimize the therapies to treat a variety of malignancies. Sadelain points out even these small studies show that different co-stimulatory molecules have different effects and, at least for CARs, some may work better for certain cancers than others. So part of the optimization process includes giving both TCR- and CAR-based T-cell therapies the optimal mix of enhancing molecules and targets to achieve the best response. Balancing components of the immune system to achieve the desired effect, Sadelain says, "is a completely new way of conceptualizing dosing in medicine."

Designing effective treatments requires finding cell-surface antigens for T cells to target without damaging normal tissue. This specificity may prove more difficult than researchers initially thought. Many antigens found in cancer are also expressed in normal tissue — HER2, for example, which is the target of the antibody-based therapy trastuzumab (Herceptin), is also expressed in heart cells. Before researchers can make progress, they must understand how extensively each candidate target is expressed in all tissues of the body.

Recent studies have highlighted what can happen when T cells unexpectedly attack normal tissue. In a clinical trial of TCR-engineered T cells, researchers at the US National Cancer Institute were targeting the cancer-specific antigen MAGE-A3 when two of their nine patients slipped into a coma and died. It turns out that the cells also recognized another member of the MAGE-A family that the researchers later discovered is expressed in low levels in brain tissue. Another type of

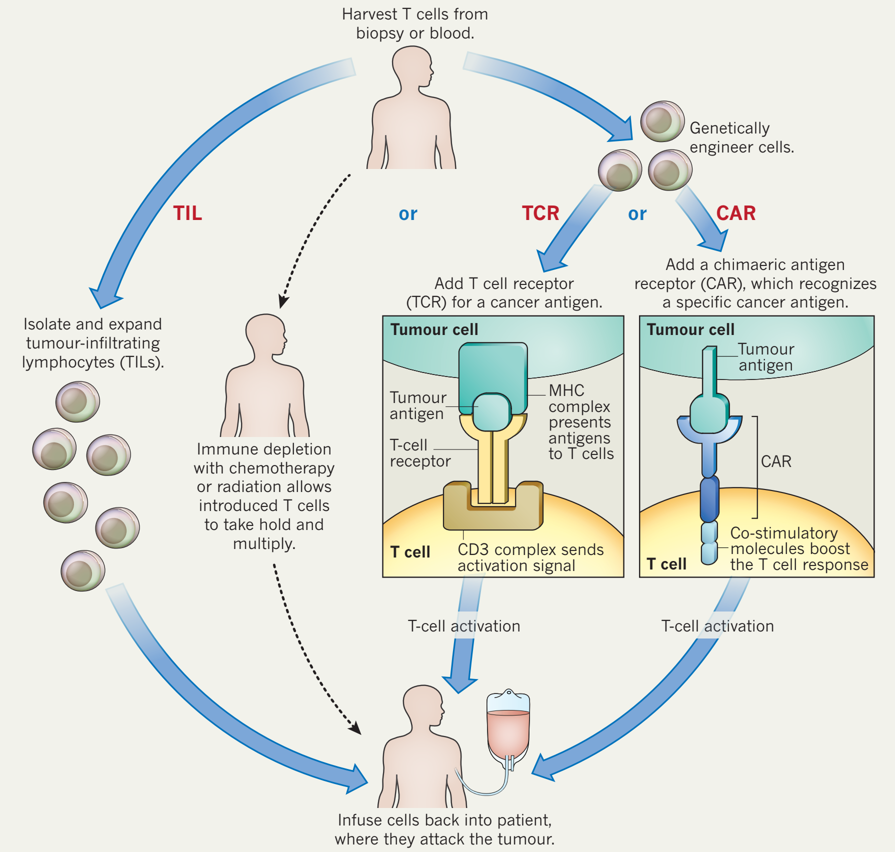
PUSHING AVAILABILITY

Adoptive cell transfer therapies for cancer are available only at a few medical centres right now, but several companies are working to change that.

Company	Therapy Type	Partnerships and trials
Adaptimmune	T-cell receptor (TCR)	Sponsoring nine pilot and phase I trials at several medical centres in the United States
Novartis	Chimaeric antigen receptor (CAR)	Licensing agreement with University of Pennsylvania to develop therapies. Establishing a global clinical trial program for CTL019, and expects to expand trials in the US next year.
Lion Biotechnologies	Tumour-infiltrating lymphocyte (TIL)	Licensing agreement with NCI to develop TIL for treating Stage IV metastatic melanoma
Kite Pharma	TCR, CAR	Agreement with the US National Cancer Institute to develop and commercialize products

CELLULAR ATTACK

Adoptive cell transfer (ACT) attacks cancer using either tumour-infiltrating lymphocytes (TILs) or genetically engineered T cells. Engineered cells are given either a new T-cell receptor (TCR) or an antibody-like molecule called a chimaeric antigen receptor (CAR); both activate the T cell when they encounter a particular cancer antigen.



MAGE-A3-specific TCR caused two patients to die from heart failure when the TCR bound to a similar protein, called Titin, which is expressed on heart cells. Adaptimmune, the company based near Oxford , UK, that developed the T-cell receptor, has implemented more extensive safety testing techniques in an attempt to prevent unexpected reactions in the future.

One of the main advantages of ACT is its speed — it works in days to weeks, much faster than other immune therapies — but triggering such a dramatic response can be dangerous. For instance, a patient with colorectal cancer who was infused with T cells as part of her CAR treatment died after experiencing an uncontrolled immune reaction called a cytokine storm. The process can also cause a condition called tumour lysis syndrome, which occurs when the chemical components of large numbers of dead tumour cells spill into the blood. “Our body is not built to get rid of 3–8 pounds of tumour,” yet ACT therapies can do this in a matter of days, says Bruce Levine, director of the University of Pennsylvania’s Clinical Cell and Vaccine Production Facility.

THE NEXT STEP

Both TCR and CAR therapies are being tested in patients with a variety of cancers, including ovarian cancer, pancreatic cancer, glioblastoma and mesothelioma, and results from these studies will help to determine whether the approach can be used more widely. Several unknowns remain, including why some patients get more therapeutic benefit from ACT than others. “Because you make the drug from a patient’s own cells, there is variation at the source,” Sadelain says. Some patients may have T cells that have lost potency or their ability to proliferate and that function more poorly. So studies must be done to find biomarkers that identify better-functioning cells, which could be used to predict patient outcomes, to sort cells before treatment, or to monitor treatment progress.

At the moment, ACT is a boutique therapy. It is performed in only a few academic medical centres worldwide, and has been tested mostly in small pilot trials in patients with advanced, chemotherapy-resistant disease. But it is progressing to larger trials. And because several groups have reproduced its success in the past few years, ACT is now drawing attention from the pharmaceutical industry (see ‘Pushing availability’). But scaling up and commercializing a therapy that needs genetically modified cells will require cheaper, faster and more automated ways to modify and grow cells than currently exist. One company at the forefront of this work is Novartis, which has invested in such a facility to help it bring the manufacturing process used at the University of Pennsylvania to larger clinical trials.

Companies are largely focused on targeting common cancer antigens, such as CD19 and MAGE-A3, but not all researchers see this as the best approach. Rosenberg, for example, believes that the most successful strategy will be one that is totally personalized: engineering cells that target antigens unique to each patient’s cancer and that are not found in healthy cells. Doing this would require extensive genetic analysis to find the tumour’s unique mutations, he says, and then custom-crafting cells to match the cancer’s genetic profile. Such an approach may be difficult, but he says that making an effective systemic cancer treatment is the priority. “Let’s find out how to cure cancer even if it’s very complex,” he says, and then find a way to simplify it to treat large numbers of patients.

As ACT therapies move closer to the mainstream, the next big step will be investigating whether and how to integrate them with other cancer immunotherapies. In December 2012, the Cancer Research Institute, a non-profit organization based in New York that funds cancer immunology, joined forces with Stand Up To Cancer, a programme of the Entertainment Industry Foundation in Los Angeles, to award US$6 million over three years to a ‘dream team’ of researchers including Yee. The aim was to find out whether adoptive cell transfer can be effectively combined with another approach that’s generating excitement in the cancer immunotherapy world: the use of immune checkpoint inhibitors. These proteins make the immune response to cancer more potent by removing signals — many of them released by tumours — that dampen the immune system (see ‘Releasing the brakes’, page S6). Pairing ACT with checkpoint inhibitors should simultaneously enhance the immune response and prime the immune system to attack the disease. “We’ve already had some preliminary data showing that the combination can be very effective,” says Yee.

Despite lingering questions, Sadelain says that scientists and clinicians are enthusiastic about the potential of adoptive cell transfer. It represents a flexible platform for cancer treatment that can be tweaked and adapted as further discoveries are made. “This is not another small molecule or antibody,” he says. “This is an entirely different approach to treating the patient.” ■

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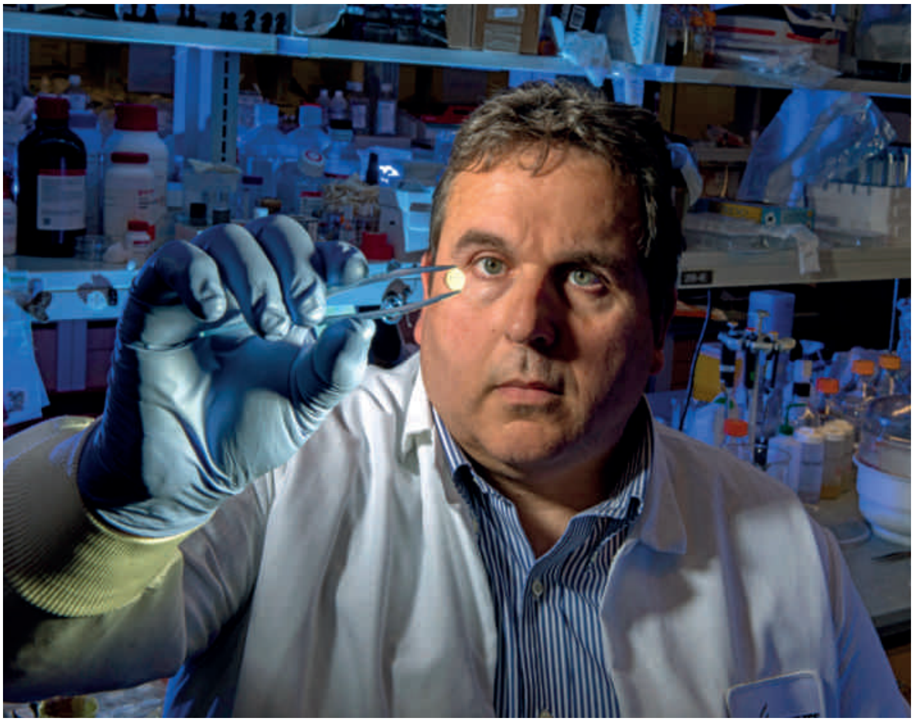

RICK FRIEDMAN

Biomaterials specialist Ed Doherty inspects a freshly made vaccine implant.

CANCER VACCINES

Material breach

An experimental vaccine implanted beneath the skin could usher in biomaterial-based immunotherapies for cancer.

BY ELIE DOLGIN

Ed Doherty ejects a tiny white disc from the hydraulic lab press in front of him and drops it into the palm of his hand. This device, about the size of an aspirin tablet, is a surgical implant that could have a huge clinical impact: it recruits and stimulates immune cells to attack tumour cells specific to an individual patient. And it is so easy to make that it could herald the future of personalized cancer-vaccine therapy. "You could have one of these set-ups at every hospital across the country," says Doherty, a biomaterials researcher at Harvard University's Wyss Institute for Biologically Inspired Engineering in Boston, Massachusetts.

Each implant contains cellular growth factors, DNA and bits of freeze-dried cells excised from a patient's own tumour, all contained within a scaffold that dissolves safely in the body over the course of about six months. This experimental vaccine does not contain living cells, which means that researchers can make multiple implants at once with just one round of manufacturing per patient. By comparison, other patient-specific cancer vaccines — such as sipuleucel-T, a cell–based vaccine marketed as Provenge by biotech company Dendreon of Seattle, Washington — take days of labour-intensive cell preparation for each round of treatment. And Provenge, the only FDA-approved therapeutic cancer vaccine, requires a processing plant to grow the required number of cells needed to stimulate an anticancer response from the immune system, whereas the implantable vaccine from Doherty and his colleagues uses a patient's own body as its immune-stimulating factory.

The disc resting in Doherty's hand has been designed to test cancer treatments in mice. But a few blocks away at the Dana-Farber/Brigham and Women's Cancer Center, Doherty's colleagues are fabricating similar devices for an experimental vaccine to treat people with advanced melanoma, the only clinical trial of its kind. "It's extremely exciting to couple materials science with the new insights into cancer immunology," says Glenn Dranoff, a cancer immunologist at the Dana-Farber who helped develop the vaccine, dubbed WDVAX. "This is one of the most exciting projects of my career."

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See how one patient is faring on a cancer vaccine:
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Beyond WDVAX, researchers are combining biology and materials science to engineer a host of delivery devices for potential cancer immunotherapies, from nanoparticles to injectable gels. Each new approach is an attempt to solve substantial problems with existing immunotherapies, such as a lack of cell specificity, dangerous side effects, and a short half-life once inside the body. The new materials-based strategies can deliver immunotherapies to specific organ systems and, at least in preclinical models, elicit more controlled and prolonged antitumour responses. "There's a trend now to rationalize the design of these systems so they can deliver different types of immune-modulating drugs in a reliable way," says Tarek Fahmy, a biomedical engineer at Yale University in New Haven, Connecticut.

So far, the most promising results of bioengineered immunotherapies come from studies on mice. In fact, data from the WDVAX vaccine in mice were so compelling[1,2] that all eyes have turned now to the human trial. "The biology and preclinical work completely make sense to go forward," says Dana-Farber oncologist Stephen Hodi, who is leading the trial. "Now, we just have to determine safety and efficacy."

As part of Hodi's phase I trial, patients with metastatic melanoma, in which the cancer has spread from its original site, began receiving the cancer-killing implants in late August 2013. The two-year trial aims to enrol 25 participants, each of whom will receive a total of four vaccine implants over the course of several months.

SCAFFOLD SPECIFICATIONS

WDVAX is the brainchild of Dranoff and Harvard bioengineer David Mooney and its strategy is predicated on recruiting and programming immune cells within the biomaterial implant. To facilitate this, the implant includes a backbone of biodegradable plastic loaded with a mix of the same three ingredients used by Doherty in his mouse prototype — a combination of dried-up tumour proteins, a growth factor and DNA molecules — all of which are brought together using a high-pressure gas foaming procedure to yield a porous scaffold into which immune cells can penetrate. The end product, says Doherty, feels like a "punched out kitchen sponge".

Now that WDVAX has proven effective in mice, it must be proven safe in humans — something the researchers believe should be straightforward. All four elements, individually, "are known to be safe", Mooney says, "and are known to be safe at much greater quantities than what we're using."

The plastic backbone is made from a polymer called polylactide-co-glycolide, which is commonly found in dissolvable stitches. The growth-factor protein, granulocyte-

macrophage colony-stimulating factor, is already sold as sargramostim (Leukine) to help cancer patients make more white blood cells; it acts by recruiting immune-system messengers called dendritic cells (see 'Evidence presenter', page S9) into the plastic structure. And the synthetic DNA molecules known as CpG oligonucleotides, which stimulate dendritic cells by mimicking a bacterial infection, have been tested extensively in clinical trials as a vaccine adjuvant.

The only novel ingredient is the patient-derived melanoma extract. This ground-up tumour biopsy serves as the antigenic material that the dendritic cells relay to other parts of the immune system, teaching it that these are foreign substances that must be eliminated. Since the melanoma extract is taken from the patients' own tumours, it should not pose a health risk, according to Mooney.

In 2009, Mooney, Dranoff and their colleagues, led by Harvard graduate student Omar Ali, showed that dendritic cells activated by the mouse version of the WDVAX implant headed directly to lymph nodes near the tumour, where they primed the immune system's T cells to kill cancerous cells, leading to tumour regression. Such a targeted approach avoided the side effects caused by systemic therapies, and it proved extremely effective in mice with an aggressive form of melanoma that normally kills the animals within three weeks. Ninety per cent of the mice that were vaccinated before tumour onset survived for at least three months[1], and about half of the animals that received two vaccine implants after the cancer had already taken hold displayed similar rates of survival[2].

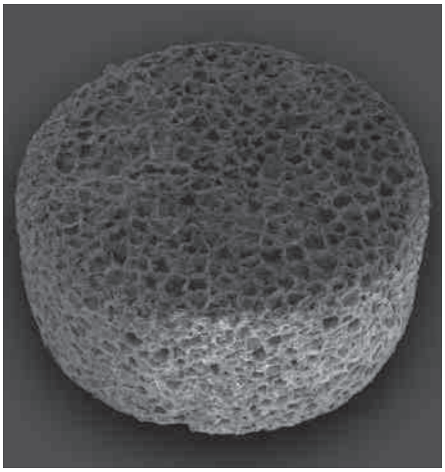
Willem Overwijk, a tumour immunologist who studies melanoma vaccines at MD Anderson Cancer Center in Houston, Texas, finds the data impressive. "The immunology is sound and the antitumour effects are pretty significant," he says. "I think there's some real possibility for this approach."

In unpublished work, the researchers have also combined their vaccine with antibodies that block either the protein cytotoxic T-lymphocyte antigen 4 (CTLA-4) or a protein called programmed death 1 (PD-1), two immune checkpoint receptors that are a major focus of the pharmaceutical industry. A single dose of the vaccine alone slowed cancer growth (but did not eliminate disease) in mice with established melanoma, and the anti-CTLA-4 or anti-PD-1 antibodies provided little benefit on their own. However, the combined approach led to tumour eradication in more than half the animals tested. "There may be some synergy between these approaches to promote tumour regression," says Mooney.

TARGET PRACTICE

Although WDVAX may be the first bioengineered immunotherapy used to treat patients, it is not the only one in development. Rather than using a device that must be surgically implanted, other researchers are engineering materials that — once injected into the body — can track down immune cells either around the tumour itself or in nearby lymph nodes.

For example, Darrell Irvine, a bioengineer at the Massachusetts Institute of Technology in Cambridge, is targeting tumours and the corresponding lymph nodes at the same time. As with WDVAX, Irvine is aiming for a treatment that is more specific than current therapies so as to kill only the malignant cells and leave healthy ones intact. He and his team tethered two molecules — both of which promote T-cell responses against many types of tumours but which can lead to inflammation — onto plastic-coated fat globules, each about 200 nanometres in diameter[3].



A vaccine implant up close and personal.

When injected directly into melanoma tissue in mice, these drug-coated nanoparticles became trapped inside the tumours and the nearby lymph nodes. Around two-thirds of the animals treated in this way experienced complete tumour regression, with no signs of the inflammatory side effects that can be lethal in this mouse model. "You're getting very potent immunotherapy stimulation, but you're avoiding toxicity because you keep the therapeutics out of the systemic circulation," Irvine says.

Similarly, Fahmy and his colleagues at Yale have engineered even smaller drug-laced nanoparticles that get trapped in the blood vessels surrounding tumours, where they release their payload to activate both the adaptive and non-adaptive arms of the immune system[4]. Meanwhile, bioengineers Melody Swartz and Jeffrey Hubbell at the Swiss Federal Institute of Technology in Lausanne made nanoparticles just 30 nanometres across, with CpG molecules anchored to their surfaces. When injected into the skin, these tiny particles migrate into the lymph nodes where the CpG adjuvant helps augment the body's natural anticancer responses[5].

"You just load that lymph node up with adjuvant," says Swartz, "and then you can activate T cells against the tumour antigens that have drained there naturally."

BEYOND MELANOMA

Now that WDVAX has moved into clinical trials for melanoma, Mooney has stepped back into the lab to see how else his implantable-vaccine design might be used.

Together with InCytu, a Lincoln, Rhode Island–based company that had licensed the technology (the licence has since been returned to Harvard, which is co-sponsoring the ongoing clinical trial), Mooney's team has shown that a vaccine containing antigens from a type of brain cancer known as glioma successfully induced tumour regression when implanted into the heads of afflicted rats[6]. Now, Mooney and Dranoff have funding to test the strategy in breast cancer, perhaps with a defined antigen like human epidermal growth factor receptor2 (HER2) instead of the extract from a patient's tumour. Although not patient-specific, such a vaccine could have the advantage of mass production. "We're beginning to explore much more broadly," Mooney says.

Because all of Mooney's vaccine implants require minor surgery, he is also exploring ways to make the strategy less invasive. For example, he's investigating a method that would swap out the polymer scaffold for a gel of porous microparticles; following injection under the skin, these particles clump together to form a sort of immune-priming depot. "This could be more easily administered than something that needs to be implanted," says Krishnendu Roy, a biomedical engineer at the Georgia Institute of Technology in Atlanta.

In his own lab, Roy has shown that this kind of gel-based vaccine, when loaded with immune-activating components, can boost antitumour T-cell activity in a mouse model of the blood cancer B-cell lymphoma[7]. "It's just an injection like anyone would take for any other kind of vaccine," he says.

Therein lies the beauty of most materials-based immunotherapies, says Mooney. The engineering may seem complex to cancer biologists, who might not be as familiar with certain technologies, but the implementation is very easy for any physician to understand. "It's stunning," he says, "just how simple this is." ■

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